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Review

Adenine nucleotide translocase 2 is a key mitochondrial protein in cancer metabolism ☆

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ABSTRACT

Adenine nucleotide translocase (ANT), a mitochondrial protein that facilitates the exchange of ADP and ATP across the mitochondrial inner membrane, plays an essential role in cellular energy metabolism. Human ANT presents four isoforms (ANT1-4), each with a specific expression depending on the nature of the tissue, cell type, developmental stage and status of cell proliferation. Thus, ANT1 is specific to muscle and brain tissues; ANT2 occurs mainly in proliferative, undifferentiated cells; ANT3 is ubiquitous; and ANT4 is found in germ cells. ANT1 and ANT3 export the ATP produced by oxidative phosphorylation (OxPhos) from the mitochondria into the cytosol while importing ADP. In contrast, the expression of ANT2, which is linked to the rate of glycolytic metabolism, is an important indicator of carcinogenesis. In fact, cancers are characterized by major metabolic changes that switch cells from the normally dual oxidative and glycolytic metabolisms to an almost exclusively glycolytic metabolism. When OxPhos activity is impaired, ANT2 imports glycolytically produced ATP into the mitochondria. In the mitochondrial matrix, the F1F0-ATPase complex hydrolyzes the ATP, pumping out a proton into the intermembrane space. The reverse operations of ANT2 and F1F0-ATPase under glycolytic conditions contribute to maintaining the mitochondrial membrane potential, ensuring cell survival and proliferation. Unlike the ANT1 and ANT3 isoforms, ANT2 is not pro-apoptotic and may therefore contribute to carcinogenesis. Since the expression of ANT2 is closely linked to the mitochondrial bioenergetics of tumors, it should be taken into account for individualizing cancer treatments and for the development of anticancer strategies. This article is part of a Special Issue entitled: Bioenergetics of Cancer.

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1. Glycolysis replaces oxidative phosphorylation in cancer cells

In normal cells, energy homeostasis is ensured through at least three metabolic pathways, i.e. glycolysis, lipogenesis and the tricarboxylic acid (TCA) cycle, all of which are closely linked to the biosynthesis of amino acids and nucleotides. Although normal cells use a variety of energy sources, glucose is the key energy source for cell growth. Glucose, taken up by the glucose transporter system, is converted to pyruvate through the glycolytic pathway [1,2]. The pyruvate, which is then converted to acetyl-CoA by pyruvate dehydrogenase, serves as a substrate for the TCA cycle in mitochondria. The mitochondria generate ATP through the TCA cycle and the oxidative phosphorylation (OxPhos). The OxPhos mechanism consists of a respiratory chain comprising of three non-reversible proton pumps (NADH dehydrogenase, ubiquinol cytochrome *c* reductase, and cytochrome *c* oxidase), an enzyme complex not involved in proton pumping (succinate dehydrogenase), and a reversible proton pump (F1F0-ATP synthase), which uses the energy of the proton gradient across the membrane to synthesize ATP [3]. The mitochondrial ATP produced is exported to the cytosol by adenine nucleotide translocase (ANT) (Fig. 1A). The respiratory complexes, F1F0-ATP synthase, and some proteins functionally bound with it in the mitochondrial inner membrane, form even more complicated structures, or supercomplexes, which promote faster diffusion of substrates or create a tunnel effect in enzymatic reactions [4]. According to Peter Mitchell's chemiosmotic theory, the energy-rich intermediate of OxPhos is the proton gradient ($\Delta\mu H^+$) across the mitochondrial inner membrane. The driving force is defined as the proton motive force (Δp), consisting of an electrical component ($\Delta\Psi m$) and a chemical component (ΔpHm) [5]. The stoichiometric efficiency of OxPhos is determined by the P/O ratio which defines the relationship between ATP synthesis and oxygen consumption. Both the rate and the efficiency of OxPhos are complex-

Abbreviations: ANT, adenine nucleotide translocase; ATPsynβ, β subunit of the F1 component of ATP synthase; $\Delta\Psi m$, mitochondrial inner membrane potential; HKII, hexokinase II; mtDNA, mitochondrial DNA; NADH+H+, reduced nicotinamide adenine dinucleotide; OxPhos, oxidative phosphorylation; VDAC, voltage-dependent anion channel

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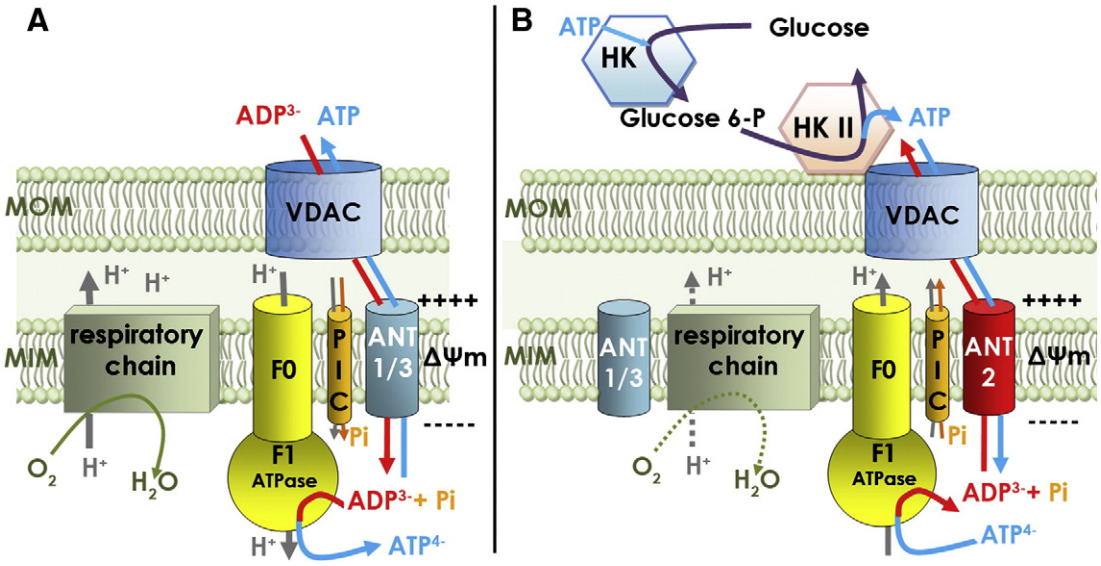

Fig. 1. Models indicating the role of ANT isoforms in oxidative phosphorylation and glycolysis. A. ANT1 and ANT3 mediate mitochondrial ATP synthesis in non-tumoral cells. Inorganic phosphate (Pi) is transported across the mitochondrial inner membrane (MIM) into the mitochondrial matrix by the mitochondrial phosphate carrier (PiC). F1F0-ATPase combines Pi and ADP to form ATP, which is then exchanged for ADP across the MIM by ANT1 or ANT3. The whole reaction is driven by a proton gradient maintained mainly by the respiratory chain. B. Aerobic glycolysis leads to defective mitochondrial ATP production in tumoral cells. The mitochondrial hexokinase isoform, HK II, generates ATP from glucose 6-P (G6P) produced by the cytoplasmic hexokinase, HK. The ATP^{4-}, imported into mitochondria across the MOM through the voltage-dependent anion channel (VDAC), and then across the MIM by the ANT2 isoform, contibutes to the maintenance of the mitochondrial membrane potential ($\Delta\Psi m$). The hydrolysis of imported glycolytic ATP^{4-} by the F1 component of ATP synthase leads to (i) the release of ADP^{3-} in the mitochondrion with the gain of a negative charge on the matrix side; and (ii) the ejection of a proton into the intermembrane space through the F0 component.

regulated by various factors, including the reductive power of the respiratory chain, i.e. the $NADH+H^+/NAD^+$ ratio, and the membrane potential ($\Delta\Psi m$), through the interaction of allosteric effectors such as ATP or ADP, i.e. the ATP/ADP ratio, with respiratory chain complexes. Active transport of cations or anions across the membrane also decreases Δp, stimulating the three proton pumps and consequently the mitochondrial respiration. In mitochondria, electrogenic transport, involving the uniport uptake of Ca^{2+} by the calcium carrier and of ADP^{3-} through antiport exchange between ATP^{4-} and ANT, decreases $\Delta\Psi m$. The electroneutral symport uptake of the negatively charged phosphate, together with an H^+ by the phosphate carrier, decreases ΔpH, and the exchange of mitochondrial aspartate against glutamate and an H^+ by the aspartate/glutamate carrier decreases Δp [6].

In most cancer cells, the rate of glucose uptake is higher and the mitochondrial oxidative phosphorylation is lower than in healthy cells [7]. This metabolic re-programming triggers a cascade of events in tumor cell physiology [8]. Increased glycolytic activity in cancer cells may be due to mitochondrial defects or adaptation to a hypoxic tumoral microenvironment [9]. The metabolism of glucose to lactate persists even in aerobic conditions, known as the 'Warburg effect', suggesting that the metabolic shift is highly regulated in tumor cells. This metabolic switch affects all the factors involved in OxPhos regulation, i.e. the P/O, $NADH+H^+/NAD^+$, ADP/ATP ratios and $\Delta\psi m$ [10–12]. Thus, although glycolytic ATP synthesis may be sufficient for cell growth, cancer cells need to maintain the $\Delta\psi m$ and mitochondrial homeostasis for Ca^{2+} exchange, ionic transport and the operation of metabolite pathways.

ANT, a mitochondrial inner membrane protein, is essential to the metabolic adaptation of cancer cells during tumoral development. Of the four ANT isoforms found in humans, ANT2 is particularly involved in the process of maintaining mitochondrial $\Delta\psi m$ and preventing apoptosis.

2. ANT regulates oxidative phosphorylation in normal cells

Adenine nucleotide translocase (ANT) is the unique catalyst of ADP/ATP exchanges across the mitochondrial inner membrane. It was the first member of the large mitochondrial carrier family to be isolated, reconstituted into liposomes, cloned, and crystallized in the form of a complex with its specific inhibitor carboxyatractyloside [13,14]. ANT, the most abundant protein of the mitochondrial inner membrane, comprises 300–320 amino acid residues forming six transmembrane helices. The functional unit of ANT, a homodimer, acts as a gated pore channeling single molecules of ADP and ATP [6]. During the transport of these molecules, ANT takes up a conformation oriented toward the cytoplasm (c-conformation) or toward the mitochondrial matrix (m-conformation) [15]. Atractyloside and carboxyatractyloside are specific inhibitors of ANT that act by fixing the c-conformation, whereas bongkrekic acid (BKA) inhibits ANT by fixing the m-conformation.

Although ANT is implicated in basal proton leaks [16] and in inducible proton leaks [17], the translocase is highly selective of the adenine nucleotide. Thus, ANT plays an essential role in cell bioenergetics by regulating the ADP/ATP ratio in mitochondrial oxidative phosphorylation.

3. The human genome encodes four ANT isoforms

The human genome presents four ANT isoforms encoded by the genes (*ANT1-4*) [18] (Table 1). ANT1, ANT2 and ANT3 are structurally similar, each gene possessing four exons, a 1.4 kb cDNA; the peptidic sequences of the three proteins encoded are about 90% identical. In contrast, the protein encoded by *ANT4* shares 66–68% identical amino acids with the other three isoforms [19], this lower homology being explained by the presence of two additional exons. Each ANT isoform possesses a specific expression depending on cell type, nature of tissue, developmental stage and status of cell proliferation. The regulatory sequences in the 5′ region differ among the *ANT*s and some specific motifs have been studied (Table 1). Most of the data concerning the expression of ANTs depend on mRNA quantifications, correlated with protein measurements. However, the results are subject to caution since the specificity of ANT isoforms is difficult to establish because of the high incidence of identical amino acids in these transmembrane proteins. Currently, no reagent allows

Table 1
Human adenine nucleotide translocase genes.

ANT isoform	Chromosome	ATP/ADP exchange	Apoptotic nature	Tissue expression	References
Genecard	Exon number			Regulatory elements	
ANT1	4q35	ATP export to the cytosol	Pro-apoptotic	Heart, skeletal muscle and brain	[18,29,30,76–78]
SLC25A4	4			CCAAT and TATA boxes	
				Oxidative regulatory OXBOX/REBOX	
ANT2	Xq24	ATP import into the matrix	Anti-apoptotic	Rapidly growing cells	[23,24,27,28,40,55,71,79,80]
SLC25A5				Cancer and cancer cell lines	
	4			TATA box, Sp1 elements (the AB boxes)	
				Go-1/Go-2, that bind nuclear factor 1 (NF1)	
				Glycolysis regulatory GRBOX	
ANT3	Xp22	Constant ATP export to the cytosol	Pro-apoptotic	Constitutively expressed in all tissues	[76,81–83]
SLC25A6	4			SP1 repeat domain; GC boxes	
ANT4	4q28.1		Anti-apoptotic	Testis and germ cells	[19,84–86]
SLC25A31	6				

satisfactory isoform-specific inhibition of ANT activity. However, ANT1 is known to be highly expressed in differentiated tissues such as skeletal muscle, heart and brain [18]. The major physiological role of ANTs is the mitochondrial export of ATP and import of ADP [20] (Table 1). The ANT3 isoform is expressed in all tissues, the expression being correlated with the activity of oxidative metabolism [18,21–23]. Although *ANT4* transcripts have been found in liver and brain tissues, ANT4 appears to be more specific to the testis and germ cells [19].

ANT2 is specifically expressed either in undifferentiated cells, such as lymphocytes, or in tissues that are able to proliferate and regenerate, such as those of the kidney and liver [18,21]. The expression of ANT2, which has been shown to be growth-dependent [24], is considered as a marker of cell proliferation [25]. The *ANT2* gene expression is down-regulated in differentiated cell lines and remains unexpressed, or only slightly expressed, in most tissues [18]. Consequently, ANT2 expression has been widely studied in cancer cells and cell lines. Unlike the ANT1 and ANT3 isoforms, ANT2 is strongly overexpressed in various types of human cancer cells and in several hormone-dependent cancers compared with normal human fibroblasts and hepatocytes [23,26,27].

4. The ANT2 isoform is specifically involved in the glycolytic metabolism of cancer cells

All mammals possess four ANT isoforms, ANT1-4 [24,28,29], except for rodents in which only three isoforms have been found, i.e. ANT1, ANT2 [30,31] and a testis isoform ANT4. Whereas Ant1$^{-/-}$ mice develop mitochondrial myopathy, the disruption of *ANT2* leads to perinatal embryonic lethality [32], suggesting that the role of ANT2 is essential to the developmental process. In yeast, the mitochondrial ADP/ATP translocase exists under three isoforms encoded by *AAC1-3* [33–35]. Under aerobic conditions, the AAC1 and AAC2 proteins ensure yeast cell growth through mitochondrial ATP synthesis and ATP/ADP exchanges. In contrast, under anaerobic conditions, AAC3 proteins are essential for the growth of yeast on a fermentable substrate [36]. The *AAC3* gene is regulated by heme through the ROX1 factor [37], which mediates oxygen repression in several other yeast genes [38]. This has led to the hypothesis that under anaerobic conditions, AAC3 provides glycolytic ATP to mitochondria [39]. Thus, in mitochondria with impaired respiration, the activity of F0F1-ATPase is reversed such that, at the expense of ATP hydrolysis, it contributes to the maintenance of $\Delta\Psi m$ at a suboptimal level by pumping protons out of the matrix. In addition, electrogenic ATP^{4-} uptake *versus* ADP^{3-} uptake into mitochondria completes the maintenance of mitochondrial $\Delta\psi m$ (Fig. 1B). When human ANT proteins are compared to yeast AAC proteins, the yeast AAC3 and the human ANT2 isoforms are the most closely related, with a 53% sequence identity [40]. Comparison of the *ANT2* and *AAC3* proximal promoters reveals a ROX1-like binding motif in the ANT2 promoter region. This motif, found neither in the other human ANT gene promoters nor in the yeast *AAC1* and *AAC2* gene promoters, is known as the glycolysis-regulated box (GRBOX). A yeast triple-null mutant, in which the three *AAC* genes were disrupted, developed under anaerobic conditions, when transformed with the human *ANT2* cDNA construct as opposed to the *ANT3* cDNA construct [40]. In endosymbionts, such as Rickettsia, which are obligate intracellular parasites, phylogenetic studies have shown that a combination of two types of ATP/ADP translocase was involved in the switch from the glycolytic mechanism (anoxia) to the oxidative metabolism (normoxia) [41]. This suggests that ANT2 may have kinetic properties allowing the import of ATP into mitochondria [23] to maintain mitochondrial $\Delta\psi m$ and specific intra-mitochondrial anabolic pathways [42] (Fig. 1B).

Cancer cells are known to have a glycolytic phenotype that favors cellular accumulation of intermediates such as lactate. An increase in the glycolytic metabolism is associated with mitochondrial OxPhos damage or inactivation due to the intrinsic conditions of transformed tumoral cells, their undifferentiated state and their microenvironment [43–45]. *In vitro* studies have shown that some tumoral cell lines maintain the ability to use their mitochondrial energetic background. Interestingly, the expression of ANT isoforms is closely related to the energetic metabolic properties of tumoral cells [46]. The induction of ANT2 expression in cancer cells is directly related to the higher glycolytic metabolism whereas ANT3 is not affected [23]. Thus, undifferentiated tumoral cell line derived from osteosarcoma overexpressing ANT2 develops in hypoxic conditions whereas the hepatocarcinoma tumoral cell line, with a more differentiated phenotype and lower ANT2 expression, was arrested at the G1/S checkpoint [47]. Under conditions of hypoxic stress, cell growth depends on the rapid metabolic adaptation to decreased mitochondrial ATP production. Oxygen deprivation leads to the arrest of the mitochondrial respiratory chain activity, causing a collapse of the mitochondrial membrane potential ($\Delta\Psi m$), resulting in cell death. Cancer cells, which can survive a complete OxPhos suppression by using the glycolytic metabolism [48], depend mainly on the ATP uptake to generate their mitochondrial $\Delta\Psi m$ [23]. It has been recently shown that the glycolytic phenotype in cancer can be altered by promoting oxidative phosphorylation with dichloroacetate, an inhibitor of pyruvate dehydrogenase kinase [49]. In human non-small-cell lung cancer, glioblastoma and breast cancer cell lines, the use of dichloroacetate increased the production of reactive oxygen species and decreased $\Delta\psi m$ leading to cell death. Similarly, a highly glycolytic cell line unable to reactivate the OxPhos metabolism was found to be more sensitive to chloroethylnitrosourea, an anticancer agent, as compared to a tumoral cell line conserving at least partial mitochondrial OxPhos activity [50]. In most transformed tumoral cells, mitochondrial ATP consumption imposes the reversal of the cellular ATP phosphoryl pathway. ANT2 is co-expressed with hexokinase II (HK II) and ATPsynβ, an essential subunit of mitochondrial F0F1-ATPase, suggesting a mechanism for the regulation of ATP import [23] (Fig. 1B). The HK II isoform was shown to be the predominantly

overexpressed form of HK in rapidly growing tumors and under hypoxic conditions [47,51]. To ensure the maintenance of energetic states in membrane-bound processes, most glycolytic enzymes are associated with the cytoplasmic membrane and the membranes of the intracellular compartments [52]. Glycolytic enzymes thus contribute to intracellular phosphoryl transfer and spatial distribution [53]. Cytoplasmic HK, which initiates glycolysis by phosphorylating glucose to glucose-6P (G6P), is inhibited by the product; in contrast, HK II, bound to the outside of the mitochondrial outer membrane, is not inhibited by G6P. According to our hypothesis, HK II, which is bound to the voltage-dependent anion channel (VDAC) located on the mitochondrial outer membrane [54] uses cytosolic G6P to provide mitochondria with ATP through an inverse reaction.

This involves the transfer of glycolytic ATP through the VDAC and ANT2 toward the matrix side of the mitochondrial inner membrane. Within the inner membrane, ANT and F0F1-ATPase associate with the inorganic phosphate carrier (PiC) to form a complex called the ATP-synthasome [55]. This complex supports a mechanism in which the release and exit of ATP/ADP and P(i) are highly localized and tightly coordinated events. The imported ATP may then be used either for the essential intra-mitochondrial enzymatic pathways or hydrolyzed to ADP by the F1 part of the F0F1-ATPase to maintain the $\Delta\Psi m$ [23].

This type of ATP hydrolysis by the reverse action of mitochondrial F1F0-ATPase has also been associated with pathological conditions when the respiratory chain is inhibited as in the ischemic phase of some neurodegenerative diseases [56–58], as well as in macrophages activated by interferon gamma and lipopolysaccharide that consequently inhibit OxPhos with NO [59]. The mitochondrial import of glycolytic ATP is essential for maintaining $\Delta\Psi m$ and preventing apoptotic cell death. Since ANT2 has been reported to be up-regulated in OxPhos-deficient liver pathology [60], a detailed investigation of the expression of ANT isoforms should prove useful in such disorders.

5. ANT isoforms play a role in cell apoptosis

The direct implication of ANT in apoptosis offers an additional reason for focusing on ANT in cancer development. It has been shown that ANT and members of the Bcl2-Bax family create contact sites between the mitochondrial inner and outer membranes. ANT cooperates with Bax, the matrix cyclophilin D (CyP-D) and the outer membrane voltage-dependent anion channel (VDAC or porin) to form a permeability transition pore (PTP), a lethal pore during apoptosis. When open, this protein complex acts as a non-specific channel, allowing molecules under 1500 Da to pass freely through the mitochondrial inner membrane [61]. The PTP is a high-conductance, Ca^{2+}-activated channel modulated by a variety of pathophysiological effectors [62,63]. Cyclosporin (Cs)A, which inhibits the matrix CyP-D peptidyl–prolyl-cis-trans-isomerase activity, desensitizes the opening of PTPs [64]. PTPs may open following a reduction of $\Delta\psi m$, leading to complete collapse of ion/solute equilibration and secondary impairment of respiration, because of the release of pyridine nucleotides and cytochrome *c*, or because of substrate depletion [65]. The release of cytochrome *c* into the cytosol is considered to be the key regulatory step that irreversibly commits cells to apoptosis. While some models suggest that cytochrome *c* release occurs in the absence of PTPs, being mediated by the pro-apoptotic protein Bax, others suggest the involvement of various components of PTPs with or without the cooperative action of Bax [66]. The precise molecular nature of the pore is still under debate since it was found that mitochondria isolated from mouse cells lacking two ANT isoforms, ANT1 and ANT2, could still undergo CsA-sensitive, Ca^{2+} induced swelling, demonstrating that the ANT is not required for this process [32]. However, the increased capacity of mitochondria devoid of ANT to accumulate Ca^{2+} ions before the opening of PTPs suggests that ANT plays at best a regulatory role in the PTP function rather than being structurally involved in the pore assembly. The physiological response observed implies the presence of additional mitochondrial carriers forming pores in the mitochondrial inner membrane, so that ATP/Mg^{2+} or ornithine/citrulline transporters may also participate in the operation of PTPs [67]. Since the expression of the ANT isoforms varies according to mitochondrial energy metabolism, these isoforms may play different roles in the apoptotic process. Although, there is no evidence indicating that ANT isoforms interact differently with the various members of the Bax and Bcl2 family, the overexpression of ANT isoforms reveals variations in apoptotic behavior. Thus, ANT1 overexpression induces rapid cell death with a concomitant decrease in $\Delta\Psi m$ and subsequent ATP depletion [68]. Similarly, the overexpression of ANT3 in HeLa cells induces apoptosis mainly through the mitochondrial pathway [69]. Thus, the overexpression of ANT1 and ANT3 may increase ATP export from the matrix and lead to mitochondrial de-energization inducing apoptosis. In contrast, several converging studies indicate that the ANT2 isoform is not pro-apoptotic [68,69].

Tumoral cell lines seem to support the overexpression of ANT2 as opposed to that of ANT1 or ANT3. By favoring the import of ATP and the so-called matricial conformation of ANT, the ANT2 isoform may be a direct inhibitor of the PTP function of ANT [70]. The maintenance of $\Delta\Psi m$ is critical to drive the import of mitochondrial proteins and control mitochondrial homeostasis. Since Bax and Bcl-2, which are mutated or deregulated in cancer cells, contribute to neoplastic cell proliferation by suppressing programmed cell death [65], ANT2 may be considered as an anti-apoptotic oncoprotein that functions as an inhibitor of mitochondrial membrane permeability.

Bearing in mind the function of ANT2, the anti-tumor strategy targeting the ANT2 transcript should aim at inhibiting the import of glycolytic ATP into mitochondria so as to decrease $\Delta\Psi m$ and increase cancer cell apoptosis. However, HeLa cells, transiently transfected with specific siRNA against human ANT2, showed no significant alteration in $\Delta\Psi m$ [27]. Nevertheless, in this study, the silencing of ANT2 expression combined with the action of lonidamine, an anti-tumor compound targeted at mitochondria that has already been used in various cancers, decreased $\Delta\Psi m$, inducing apoptotis. The synergistic action of lonidamine and ANT2 depletion on $\Delta\Psi m$ suggests that the exclusive reduction of the ANT2 protein expression level may not have been sufficient. In another study on human breast cancer cells, prolonged inhibition of ANT2 expression by shRNA led to the disruption of $\Delta\Psi m$ exclusively through ANT2 inhibition, inducing apoptotic cell death [71]. In human ADF glioblastoma cells, in which ANT2 is more abundant than ANT1, the silencing of ANT1 but not of ANT2 by siRNA increased the oxidative stress leading to cell death. In these astrocytic tumors, the effect of ANT1-silencing on ADF may be mediated by the loss of the ANT1 uncoupling function [72].

6. MtDNA mutations and dysregulation of ANT isoforms may lead to neoplastic transformation

Although the mitochondrial DNA (mtDNA) encodes only a small fraction (13/1500 proteins) of the mammalian mitochondrial proteome, many mitochondrial diseases are due to mtDNA mutations or to the failure to maintain mtDNA levels [73]. The deoxyribonucleoside triphosphate (dNTP) pools that serve the mitochondrial genome are synthesized primarily in the cytosol before selective import into the matrix. The intracellular bulk of dNTP is regulated to meet the specific metabolic demand for DNA precursors during the different phases of the cell cycle, whereas mitochondrial DNA replication proceeds uniformly all through the cell cycle and even during the entire lifetime of non-cycling cells. Hence, the need for the constant presence of precursor pools for mitochondrial DNA [74]. Mutations in the gene encoding the ANT1 isoform-specific to the heart muscle have been linked to progressive external ophthalmoplegia, a condition in which mtDNA undergoes multiple deletion mutations [75]. The concentrations of dNTPs at replication sites are important determinants of both the rate and fidelity of DNA replication. Since

ADP is a substrate for ribonucleotide reductase, an ANT1 deficiency might cause an imbalance in the mutagenic dNTP pool.

Dysregulation of nucleotide biosynthesis and distribution within the cell often results in neoplastic transformation [1]. Rapidly proliferating cancer cells require ample, balanced supplies of nucleotides for survival and growth. When this balance is perturbed, the risk of additional DNA mutations rises, augmenting the malignant characteristics of tumor cells. Several key enzymes involved in nucleotide biosynthesis and modification, including thymidylate synthase, ribonucleotide reductase, and DNA polymerase, are markedly up-regulated in many types of cancer and therefore considered to be promising targets for cancer therapy [1].

The dysregulation of ANT isoforms in cancer suggests that ANT may play a role in neoplastic transformation by affecting nucleotide metabolism and the integrity of the mitochondrial genome. In fact, mtDNA mutations have been reported in renal adenocarcinoma, colon cancer, astrocytic tumors, and a variety of other tumors such as those of the head and neck, thyroid, breast and prostate [3]. MtDNA mutations, which inhibit OxPhos activity by impeding the flow of electrons down the respiratory chain, may increase ROS production and contribute to neoplastic transformation.

7. Conclusion

The metabolic changes following the switch from the dual oxidative and glycolytic metabolism in normal cells to a mainly glycolytic metabolism plays a significant role in the survival and proliferation of cancer cells. Further investigation of the glycolytic phenotype, an early manifestation of carcinogenesis, should offer novel therapeutic approaches. Thus, the silencing of ANT2 so as to promote cell death, or the sensitization of tumoral cells to apoptotic agents could prove useful in anticancer treatment. The chemotherapeutic efficiency may depend on the capacity of cancer cells to adjust the energy balance between glycolysis and oxidative phosphorylation. The ANT2/ANT3 expression ratio may be used to predict whether the up-regulation of glycolysis or the activation of OxPhos would be more effective in maintaining mitochondrial $\Delta\Psi m$. In any case, the mitochondrial bioenergetic background of tumors should be taken into account for the conception of individualized cancer treatments and for the development of anticancer strategies.

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